ANEMIA AND IRON DEFICIENCY IN CHILDREN WITH POTENTIAL CELIAC DISEASE

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Abstract

Objectives: Active screening for celiac disease frequently detects seropositive children with normal villous morphology (potential celiac disease). It remains unclear whether these subjects should be treated. We here investigated the prevalence of anemia and iron deficiency in children with potential and mucosal atrophy celiac disease.

Methods: The prospective study involved 19 children with potential disease, 67 with partial or subtotal villous atrophy (P/SVA) and 16 with total villous atrophy (TVA). Twenty-three healthy children comprised the control group. The groups were compared for various clinical, histological and laboratory parameters and hepcidin.

Results: The prevalence of abnormal parameters was as follows (controls, potential celiac disease, P/SVA and TVA, respectively): anemia 0%, 15%, 22% and 63%; low iron 5%, 0%, 14% and 50%; increased transferrin receptor 1 (TfR1) 5%, 16%, 20% and 47%; low ferritin 0%, 21%, 35% and 87%; and low transferrin saturation 10%, 11%, 41% and 71%. One subject had low folate and none had low vitamin B12. The median values for hemoglobin, total iron, ferritin and transferrin saturation were significantly lower and TfR1 values higher in TVA group compared with other groups. After a median of seven months on a gluten-free diet hemoglobin, total iron, ferritin and albumin in children with P/SVA exceeded the baseline values in the potential celiac disease group.

Conclusions: The development of anemia and iron deficiency in celiac disease is a continuum and may already be present in children with normal villous morphology, advocating an early diagnosis and possible dietary treatment of these patients.

Key words: Hepcidin, hemoglobin, gluten-free diet, mucosal damage

What is known

- An increasing number of children with positive celiac autoantibodies but morphologically normal small-bowel mucosa are detected by active case-finding and screening
- It remains unclear whether subjects with this so-called potential celiac disease should be treated with a gluten-free diet

What is new

- Celiac disease-associated anemia and iron deficiency is a continuum and may appear in seropositive children even before morphological villous damage
- These children may benefit of an early diagnosis and dietary treatment

Introduction

Celiac disease is a life-long disorder characterized by a heterogeneous clinical picture.[1] Recent screening studies have revealed the prevalence to be as high as 1-2% (2, 3). The only treatment for the condition is a permanent gluten-free diet, which usually results in beneficial clinical, histological and serological response (4). In all current criteria the diagnosis is still based on the morphological damage of the small-bowel mucosa (6-8), although in the latest pediatric guidelines the actual biopsy is not necessary in all cases (5). However, accurate serological tests measuring antibodies against transglutaminase 2 (TG2-ab) and endomysium (EmA) are nowadays available (5,9,10). Widespread utilization of these tests has resulted in the identification of an increasing number of seropositive subjects who still have morphologically normal mucosa and are thus not diagnosed (2, 11). Recent studies have produced a growing body of evidence that this so-called potential celiac disease may in fact already cause gluten-dependent symptoms before villous atrophy with crypt hyperplasia, the end-stage of the disease, develops (11-13). Further, most of these cases will eventually develop atrophy when continuing on a gluten-containing diet (12, 14-16). Interestingly, clinical experience suggests that children with potential celiac disease evince signs of anemia or subclinical iron deficiency. Besides a role in oxygen transport, iron plays a crucial part in many biological functions such as energy production, DNA synthesis and cell proliferation, and iron deficiency can impair psychomotor and cognitive development and lead to a defective immune system (17-21). Thus, if abnormal iron parameters may be present already in children with potential celiac disease, this would strongly support active diagnosis of seropositive patients even before the development of advanced mucosal lesion.

The aim of the present prospective study was to investigate the prevalence of anemia and abnormal iron parameters in children with potential and mucosal atrophy celiac disease. Further, these variables and a variety of other clinical and laboratory features were

compared between these seropositive patients with different stages of mucosal damage and healthy controls.

Materials and methods

Patients and study design

The study was conducted at the Tampere Centre for Child Health Research, University of Tampere and Tampere University Hospital. It involved consecutive children (age <16 years) referred to our tertiary referral center due to celiac disease suspicion and evincing positive celiac disease serology. Exclusion criteria were study refusal and negative or lacking serology. All participants underwent a thorough clinical examination and blood sampling for serology and laboratory parameters and for celiac disease genetics. Gastrointestinal endoscopy with duodenal biopsies was performed under general anesthesia. Children with established villous atrophy with crypt hyperplasia received a celiac disease diagnosis and were placed on a gluten-free diet, while those with positive autoantibodies but morphologically normal villi continued on a normal diet and comprised the potential celiac disease group (for details see below). Children with potential celiac disease were monitored every 3-6 months during the study period (up to 2.5 years) and a new endoscopy was performed in case of marked increase in the antibody values or worsening of the symptoms. Finally, all study parameters were compared between the study groups at the time of diagnosis. After the patients with a celiac disease diagnosis had been on a gluten free diet for a minimum of three months, the values of iron parameters were re-measured. Twenty-three healthy children with no celiac disease suspicion and negative EmA and TG2-ab comprised the non-celiac control group for comparisons of the study parameters. These children had participated as family members in a celiac disease screening study in which blood samples

were drawn for laboratory parameters and celiac disease serology and genetics as previously described (10).

Written informed consent was obtained from all study participants and/or their parents according to the Helsinki Declaration. The study protocol and patient recruitment were approved by the Ethics Committee of the Pirkanmaa Hospital District.

Clinical Evaluation

The clinical presentation of celiac disease was recorded and categorized into gastrointestinal symptoms (e.g. diarrhea, stomach pains, constipation, bloating), extra-intestinal symptoms (e.g. neurologic symptoms, rash, poor growth, anemia, fatigue, arthralgia) or screen-detected in at-risk groups (e.g. celiac disease in relatives or previous type 1 diabetes). In addition, family history of celiac disease and presence of disease-associated (e.g. type 1 diabetes, autoimmune thyroidal disease) or other chronic conditions were recorded. Height and body mass index (BMI) at celiac disease diagnosis were expressed as standard deviation scores (SDS) for age and sex.

Small-Bowel Mucosal Histology and IgA deposits

A minimum of five small-bowel mucosal biopsies were taken from the distal duodenum and three biopsies from the anatomical duodenal bulb. The paraffin-embedded specimens were cut, stained with hematoxylin and eosin and evaluated by experienced pathologists. Only correctly oriented histological specimens with complete villus-crypt units and longitudinally cut crypts were accepted for microscopic analyses (22). The specimens were further graded on the basis of the histological findings as follows: morphologically normal villi with or without mucosal inflammation (potential celiac disease group, equivalent to Marsh 0-1 or Corazza-Villanacci A), normal villi with crypt hyperplasia (Marsh 2, Corazza-Villanacci A),

subtotal and partial villous atrophy (P/SVA group, Marsh 3a-b, Corazza-Villanacci B1) and total villous atrophy (TVA group, Marsh 3c, Corazza-Villanacci B2) (23, 24).

At least one biopsy from both the distal duodenum and the bulb area were freshly embedded in optimal cutting temperature compound (OCT) (Tissue-Tec, Miles Inc, Elkhart, IN, USA) and snap-frozen in liquid nitrogen. Staining of intraepithelial lymphocytes (IELs) was performed with 5-µm-thick frozen biopsy sections. The mucosal CD3+ IELs were stained with monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA, USA) and T-cell receptor-γ antibody (Endogen, Woburn, MA, USA) was used to stain γδ+ IELs. A 100x flat field light microscope objective was used to count positive IELs and counted cells were expressed as cells/mm of epithelium (25). Celiac disease-specific mucosal TG2-targeted autoantibody deposits (IgA deposits) were measured by direct immunofluorescence from the frozen specimens as previously described (26, 27). In celiac disease the deposits are found along the villous and crypt epithelium and around mucosal vessels, whereas in healthy individuals IgA is detected only inside plasma and epithelial cells (27).

Celiac Disease Serology and HLA Genotype

Serum IgA class EmA titers were measured by indirect immunofluorescence (in-house) with human umbilical cord as substrate. A dilution ≥ 1:5 was considered positive and positive sera were further diluted 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000. The EliA Celikey test (Phadia, Uppsala, Sweden) was used to determine serum TG2-abs. Cut-off for TG2-ab positivity was >7.0 U/l according to the manufacturer's instructions. Human leucocyte antigen (HLA) DQ2/DQ8 genotyping was performed using the SSP low-resolution kit (Olerup SSP AB, Saltsjöbaden, Sweden).

Laboratory Parameters and Hepcidin

The following associated laboratory parameters were measured by standard methods: hemoglobin (Hb, reference value (Rf) from 100-141 g/l to 130-160 g/l depending on age and sex), plasma transferrin receptor 1 (TfR1, Rf from 1.6-5.2 mg/l to 2.0-6.8 mg/l), serum total iron (Fe, Rf 6-25 µmol/l), plasma ferritin (Rf >10 µg/l), transferrin iron saturation (Rf 15-50 %), serum folate (Rf 10.4-42.4 nmol/l) and serum vitamin B12 (Rf 140-490 pmol/l). Serum bioactive hepcidin (hepcidin-25) levels were measured with parallel samples using a commercial solid-phase enzyme-linked immunosorbent assay (EIA-5258, DRG Diagnostics, Marburg, Germany). Furthermore, values for plasma albumin (Rf from 35-46 g/l to 37-51 g/l depending on age and sex), plasma alkaline phosphatase (ALP, Rf from 115-460 U/l to 80-445 U/l), plasma alanine aminotransferase (ALT, Rf <40 U/l) and plasma thyroid-stimulating hormone (TSH, Rf 0.27-4.2 mU/l) were measured by standard methods. Laboratory parameters other than iron were measured in order to further elucidate the overall severity of the disease in each group.

Statistics

Clinical characteristics and prevalence of abnormal blood parameters are presented as percentage distributions. The skewedness of the quantitative data was assessed by the Shapiro-Wilk method and most of the variables were not normally distributed. For simplicity, all data are thus expressed as medians with quartiles or with range. Laboratory values between groups were compared using the Kruskal-Wallis one-way analysis of variance or by Fisher's exact test. Changes within the groups on a gluten-free diet were compared using either the paired t-test or Wilcoxon signed-rank test as appropriate. Chi-squared test or Fisher's exact test were used to compare the proportions of abnormal laboratory parameters between the groups. P-values <0.05 were considered significant.

Results

Altogether 102 children participated the study. Of them TVA was detected in 16, P/SVA in 67, and potential celiac disease in 19 subjects. None presented with crypt hyperplasia and normal villi (Marsh 2). There were no differences in age or gender between the groups, except a trend with a borderline significance (P=0.062) for lower number of girls in the control group (Supplemental Table 1, supplemental digital content). Gastrointestinal presentation was more common in the TVA than in the P/SVA or potential celiac disease, while the latter two groups comprised more screen-detected children and those with extra-intestinal presentation. Associated conditions and family history of celiac disease were more common in potential celiac disease. By definition, all 102 patients with celiac disease suspicion and none of the controls had positive TG2-ab and/or EmA. Also, HLA DQ2/8 was present in all seropositive children and 59% of the controls (Supplemental Table 1, supplemental digital content).

Both EmA and TG2-ab differed significantly between the study groups, as their concentrations increased parallel with the severity of mucosal damage (Table 1). Similarly, the densities of CD3+ and γδ+ IELs increased gradually from potential celiac disease to TVA. There were no differences between the groups in any of the non-iron related laboratory parameters or in height and BMI-SDS, but in ALT there was an increase towards significantly higher values in TVA. TG2-targeted mucosal IgA deposits were positive in all seropositive children except two with P/SVA and one with potential celiac disease; in the three negative cases there was possible patchy distribution of the deposits (Table 1).

Anemia, low iron, high TfR1, low ferritin and decreased transferrin saturation were more common in children with advanced histological damage (Table 2). Nevertheless, anemia and abnormal TfR1, ferritin and transferrin saturation were also present in a number of subjects with potential celiac disease (Table 2). Furthermore, all these laboratory abnormalities except low iron were more common in potential celiac disease group than in

controls. Of the three potential celiac disease patients presenting with anemia one had Marsh 0 (CD3+ IELs 25 cell/mm) and the others Marsh 1 lesion, but all had increased γδ+ IELs (10.5 cells/mm, 25.1 cells/mm and 12.6 cells/mm) and positive EmA (1:200, 1:50 and 1:100) and IgA deposits. Their ferritin values were 28 µg/l, 6 µg/l and 6 µg/l. Besides these three with anemia, two potential celiac disease patients (Marsh 0, EmA 1:50 and Marsh 1, EmA 1:200) had high TfR1 (6.5 mg/l and 5.1 mg/l) and low/borderline low ferritin (7 µg/l and 10 µg/l) and transferrin iron saturation (14.2% and 17.3%). These children were between 3.5-7.2 years of age, except one girl age of 15 years of whom there was no information of excessive or prolonged menstruation, and no other pathological or dietary causes of anemia except potential celiac disease were detected. A low folate value was seen only in one patient with SVA and low vitamin B12 in none of the study children (Table 2).

The median values for hemoglobin, total iron, ferritin and transferrin iron saturation were significantly lower and TfR1 higher in TVA group compared with the other groups (Figure 1). In contrast, there were no differences in any of these parameters between the potential celiac disease and P/SVA groups, and both had significantly lower ferritin than the control group. There were no differences between the study groups in the median hepcidin levels (Figure 1). Furthermore, there were no differences in hepcidin concentrations between subgroups with or without anemia, or with or without low ferritin (data not shown). In the clinical presentation between the potential celiac disease children with or without anemia there were no significant differences as 78% of those presenting with anemia/iron deficiency suffered from gastrointestinal or extraintestinal symptoms while the corresponding figure was 90% in those with normal hemoglobin and iron parameters. There were also no correlations between hepcidin and hemoglobin or ferritin values (data not shown).

After an average of seven (range 3-13) months on a gluten-free diet the hemoglobin, TfR1, total iron, ferritin and albumin values improved significantly within both

the TVA and the P/SVA groups (Figure 2). When these follow-up values were compared with the baseline values in the potential celiac disease group, albumin in TVA group (p=0.037) was found to be significantly higher. A similar but non-significant trend was also seen in P/SVA group in total iron, ferritin and albumin (Figure 2). Anemia was still present in one (2.5%) of the P/SVA patients and none of the TVA patients in whom it was re-evaluated while on the dietary treatment.

Three children with potential celiac disease started a gluten-free diet while still having normal villi. One of them was anemic at baseline and while on diet had increased hemoglobin and total iron values. Another child experienced similar improvements even though he was not anemic at baseline. In the third case there were no marked changes in the laboratory values. All but two of the remaining 16 children have remained seropositive during a follow-up of up to 2.5 years. Furthermore, two potential celiac disease children with anemia started an iron substitution without gluten-free diet and also experienced improved hemoglobin and iron parameters.

Discussion

The main finding in the present prospective study was that, even if more common in children with severe mucosal atrophy, celiac disease-associated anemia and iron deficiency is a continuum and may appear even before morphological villous damage. Further, there were no significant differences in the iron parameters between the seropositive subjects with diagnostic PVA or SVA (Marsh 3a-b) and those with the currently non-diagnostic potential celiac disease (Marsh 0-1).

Anemia and iron deficiency are among the most common abnormal findings in untreated celiac disease (28). Accordingly, anemia was seen here in approximately one fifth of children with P/SVA and two thirds of those with TVA. Notwithstanding this correlation

with the severity of the histology, it is noteworthy that anemia was also present in 15% of the otherwise healthy potential celiac disease patients with no other obvious reason for the low hemoglobin. Moreover, while still evincing normal villi these seropositive children also had abnormalities in many other iron parameters more often than controls, of whom almost none presented with even subclinical anemia. This issue has not hitherto been investigated systematically, but a few earlier studies have in fact pointed in the same direction. In a prospective Italian study by Tosco and colleagues none of the 106 children with potential celiac disease had anemia at baseline, but four presented with low ferritin, and during a follow-up of up to three years on a gluten-containing diet four developed anemia (14). In our previous adult study three out of 11 seropositive subjects with mild enteropathy (Marsh 1-2) presented with malabsorption or anemia (29), this again being in line with the present findings.

Compatible with the proportion of abnormal iron parameters, also the absolute values were inferior in the TVA group compared with the other three groups. Notably, however, there were no significant differences between potential celiac disease and P/SVA, while in contrast both groups had lower median ferritin than the control children. In addition, on a gluten-free diet several of the iron parameters within the P/SVA and TVA groups improved to levels even better than those in the potential celiac disease patients on a normal gluten-containing diet, suggesting that the latter group already had sub-optimal values. In line with this we have previously observed improved hemoglobin values in EmA-positive children and adults who had mild enteropathy (Marsh 1-2) and were placed on an experimental gluten-free diet for one year (11, 12). These results demonstrate that, despite the differences in histology and serology, the distinction between potential celiac disease and in particular P/SVA is at least partly artificial and based more on simplified histological classification than on the actual biological nature of the disease.

Somewhat surprisingly we found neither differences in hepcidin values between the groups nor correlations between hepcidin and other iron parameters. Hepcidin is the key player in iron metabolism, as it regulates both intestinal absorption and release of iron from the body storages (18). Hitherto only a few studies have investigated hepcidin in the context of celiac disease. Bergamaschi and co-authors also found no correlation between hepcidin and anemia or other iron parameters in adults (30). However, they measured the less accurate pro-hepcidin instead of the actual functional hepcidin-25 used here (30, 31). In a sole pediatric study (only abstract available) the majority of children with villous atrophy celiac disease had low hepcidin, but in 20% it was increased and the authors speculated that they had anemia of chronic disease (32). This type of anemia is associated with high levels of pro-inflammatory cytokines, as seen for example in inflammatory bowel disease, in which a correlation between anemia and high hepcidin values has been observed (21, 33). Interestingly, although no differences between the groups were found in our study, there was a wide variation in hepcidin values, indicating that anemia in individual patients is caused by variable factors, as previously shown (34). Furthermore, the fact that two children here presented with anemia or low ferritin despite apparently completely normal duodenal mucosa (Marsh 0) suggests that iron metabolism might be disturbed even before marked intestinal inflammation. Indeed, Borrelli and colleagues (35) recently demonstrated activation of different immunoregulatory reactions even in such early potential celiac disease which could possibly affect iron metabolism (36). This could also explain the changes observed in sensitive iron parameters, especially the significantly lower ferritin values in potential celiac disease group compared with the controls (37). Further studies of the mechanisms of anemia in early developing celiac disease are evidently needed.

Currently the diagnosis of potential celiac disease remains unclear. For example, in the new ESPGHAN guidelines measurement of γδ+ IELs and IgA deposits in patients with

Marsh 1 is suggested, but there is no conclusion as to their diagnosis (5). As a result of active screening for celiac disease the number of these seropositive individuals is constantly rising and, in consequence, also their natural history has been increasingly investigated (12, 14, 38-40). In two recent Italian studies symptomatic children with potential celiac disease were placed on a gluten-free diet, while the rest continued on a normal diet (14, 38). All patients on diet became seronegative and most had a positive clinical response, while after nine years 67% of those on gluten still had normal villi (14, 38). None of the 16 children continuing on a gluten containing diet in the present study have yet developed mucosal atrophy, although control endoscopy was not done systemically to all. This is in contrast with our previous study where this was in most cases already seen after one year (12). However, the children in the earlier study had more advanced clinical and histological disease at baseline. It is also noteworthy that two of the potential celiac disease patients here seroconverted to negative. Such a fluctuation in low antibody levels has been shown before, especially in asymptomatic cases (39, 41). In these circumstances additional evidence of celiac disease, such as the appropriate genetics, positive EmA, IgA deposits and refined antibody tests are of particular importance (40-42). In sum, it is evident that the presentation and natural history of potential celiac disease is variable, but it would appear that a part of these subjects may present with clinical symptoms and signs and benefit from early dietary therapy. Considering the essential role of iron in human health, our further support at least a possibility of an early diagnosis and treatment of these children.

The major strengths of the present study are the prospective design and the variety of clinical, histological and serological parameters measured. Furthermore, particular attention was paid to the precise histomorphological analysis of the biopsies and thus correct histological grouping of the participants. The main limitation is the relatively small number of children with potential celiac disease which hampers statistical evaluation. In addition, since

currently it is not recommend to set an official diagnosis in children with potential celiac disease in Finland, it was not possible to systematically evaluate the effect of a gluten-free diet on their hemoglobin and iron parameters. Since the main aim of the study was not to investigate the natural history of potential celiac disease patients the overall duration of the study was rather short, and obviously careful long-term follow-up of these cases is necessary. It must also be mentioned that the follow-up values of iron parameters in P/SVA and TVA patients were not systemically collected from all patients who had normal values at the time of celiac disease diagnosis. Finally, the number of control children was rather small, even though we believe that they were a representative of the Finnish population in respect of iron parameter levels (43). Further, as these children belong to families where celiac disease is present they also have an increased risk for the condition and, on the other hand, possibly reduced daily gluten consumption.

In conclusion, we showed that the development of anemia and subclinical iron deficiency in celiac disease is a continuum and may already appear in seropositive children with morphologically normal mucosa. These findings support early diagnosis and either careful follow-up or alternatively active dietary treatment of children with potential celiac disease.

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Figure legends

Figure 1. Hemoglobin (A), transferrin receptor 1 (B), total iron (C), ferritin (D), transferrin iron saturation (E) and hepcidin (F) values in 23 healthy children, in 19 children with potential celiac disease (potential CD), in 67 children with partial or subtotal villus atrophy (P/SVA) and in 16 children with total villus atrophy (TVA). The values are presented as medians with quartiles (boxes) and range (whiskers). Only significant differences between the groups are shown (brackets).

Figure 2. Hemoglobin (A), transferrin receptor 1 (B), total iron (C), ferritin (D) and albumin (E) in 19 children with potential celiac disease (potential CD), 67 children with partial or subtotal (P/SVA) and 16 children with total villus atrophy (TVA) at the time of endoscopy and after an average of seven months on a gluten-free diet (only P/SVA and TVA). The values are shown as medians and quartiles. Only significant changes within the groups are shown (brackets).

Supplemental Table 1. Supplemental digital content. Baseline characteristics in 19 children with potential celiac disease, 83 with villous atrophy celiac disease and in 23 non-celiac controls.

Captions in the text:

“There were no significant differences in age or gender between the groups (Supplemental Table 1, supplemental digital content).”

“By definition, all 102 patients with celiac disease suspicion and none of the controls had positive TG2-ab and/or EmA. Also, HLA DQ2/8 was present in all seropositive children and 59% of the controls (Supplemental Table 1, supplemental digital content).”

Table 1. Baseline characteristics in 19 children with potential celiac disease, 83 with villous atrophy celiac disease and in 23 non-celiac controls.

	Potential celiac disease, n=19	Celiac disease n=83		Non-celiac controls, n=23
		P/SVA, n=67	TVA, n=16	
	%	%	%	%
Age, median (range), years	6.3 (3.5-16.9)	7.5 (1.6-15.2)	6.1 (3.7-15.6)	6.0 (2.1-11.4)
Girls	74	73	69	44
Clinical presentation				
Gastrointestinal[1]	72	75	94	ND
Extra-intestinal[2]	17	22	6	ND
Screen-detected[3]	11	3	0	ND
Celiac disease in family	72	47	33	ND
Associated disease[4]	10	1	6	ND
Other chronic disease[5]	26	37	50	ND
Positive EmA/TG2ab	100	100	100	0
HLA DQ2/8	100	100	100	59

[1]Diarrhea, stomach pains, constipation, bloating
[2]Rash, dizziness, retarded growth, anemia, iron deficiency, leg pains, tiredness, arthralgia
[3]Family risk of celiac disease, previous type 1 diabetes
[4]Type 1 diabetes, autoimmune thyroidal disease
[5] Rheumatoid arthritis, allergies, congenital glaucoma, asthma, atopic dermatitis, anorexia nervosa
ND, no data; HLA DQ2/8, celiac disease-associated human leucocyte antigen

Table 2. Baseline laboratory parameters, anthropometric data and small-bowel mucosal histology in 19 children with potential celiac disease and 83 with villous atrophy celiac disease. Values except transglutaminase 2 targeted IgA deposits are shown as medians with lower and upper quartiles.

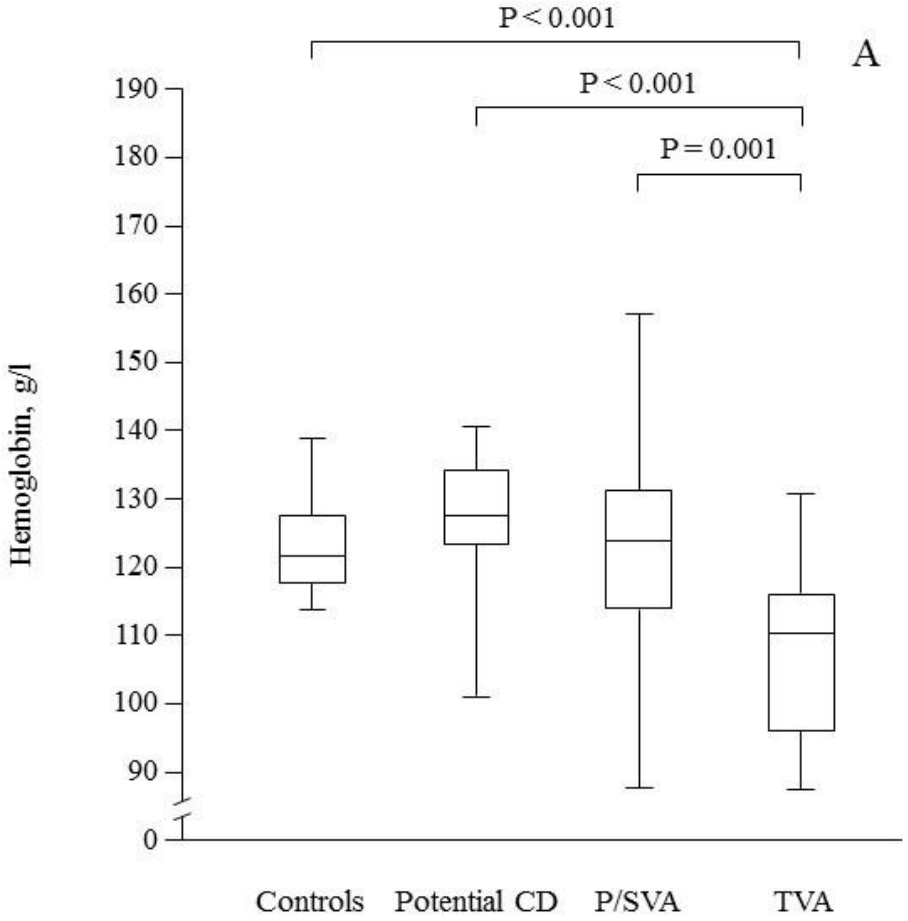
	Potential celiac disease n=19	Celiac disease n=83		P value
		P/SVA n=67	TVA n=16	
EmA, titer	1:50 (1:5, 1:100)	1:500 (1:100, 1:2000)	1:1500 (1:500, 1:4000)	**<0.001**
TG2 antibodies, U/l	24.5 (10.3, 33.0)	70.0 (21.0, 120.0)	120.0 (120.0, 120.0)	**<0.001**
Albumin, g/l	38.0 (36.5, 39.5)	38.0 (35.0, 40.0)	37.0 (36.0, 39.0)	0.517
ALP, U/l	225 (182, 265)	206 (135, 222)	214 (128, 237)	0.429
ALT, U/l	14.0 (12.0, 17.5)	17.0 (14.0, 24.3)	21.5 (16.5, 34.5)	**0.032**
TSH, mU/l	3.2 (2.7, 3.9)	2.1 (1.6, 3.2)	3.7 (2.95, 7.2)	0.323
Height, SD	-0.4 (-1.6, 0.3)	0.0 (-1.1, 0.8)	-0.4 (-0.7, 0.9)	0.178
BMI, kg/m^2	16.0 (15.3, 21.2)	11.2 (14.6, 17.7)	17.3 (15.9, 18.5)	0.768
CD3+ IELs, cells/mm	46 (24, 61)	92 (75, 111)	105 (95, 151)	**<0.001**
γδ+ IELs, cells/mm	22.0 (9.4, 35.1)	39.5 (25.9, 54.6)	34.4 (21.9, 52.5)	**0.002**
Positive IgA deposits, %	94	97	100	0.576

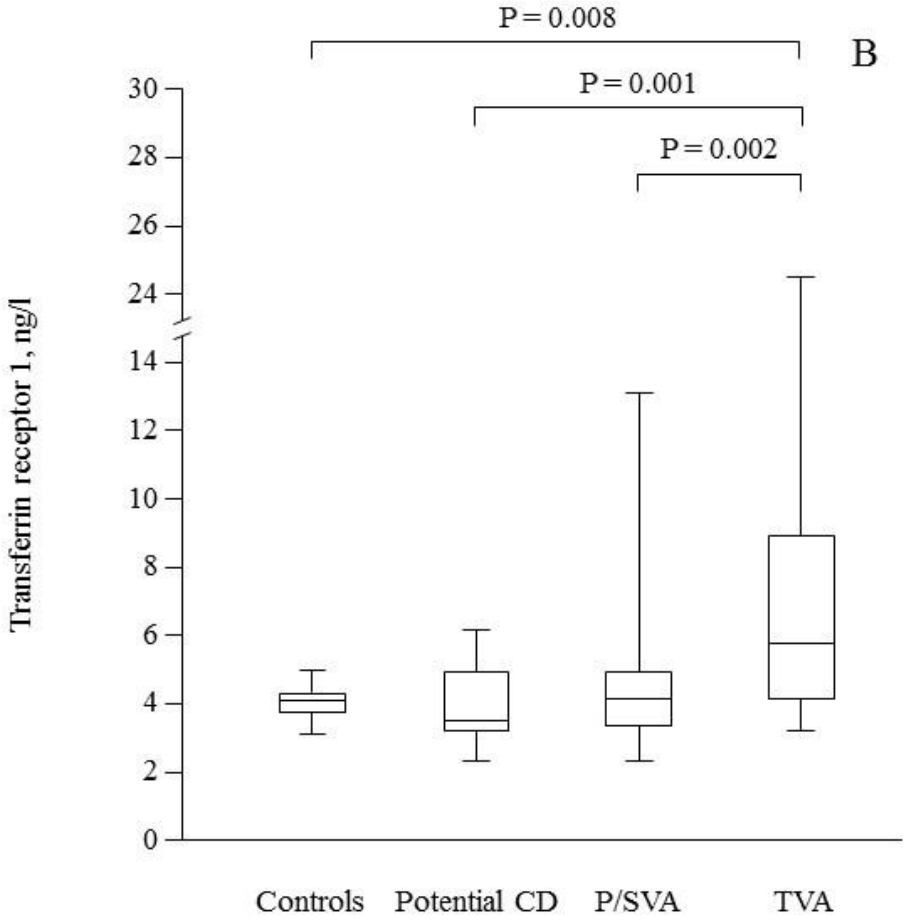
EmA, endomysial antibodies; TG2, transglutaminase 2; ALP alkaline phosphatase; ALT, alanine aminotransferase; TSH, thyroid-stimulating hormone; BMI, body mass index; IEL, intraepithelial lymphocyte

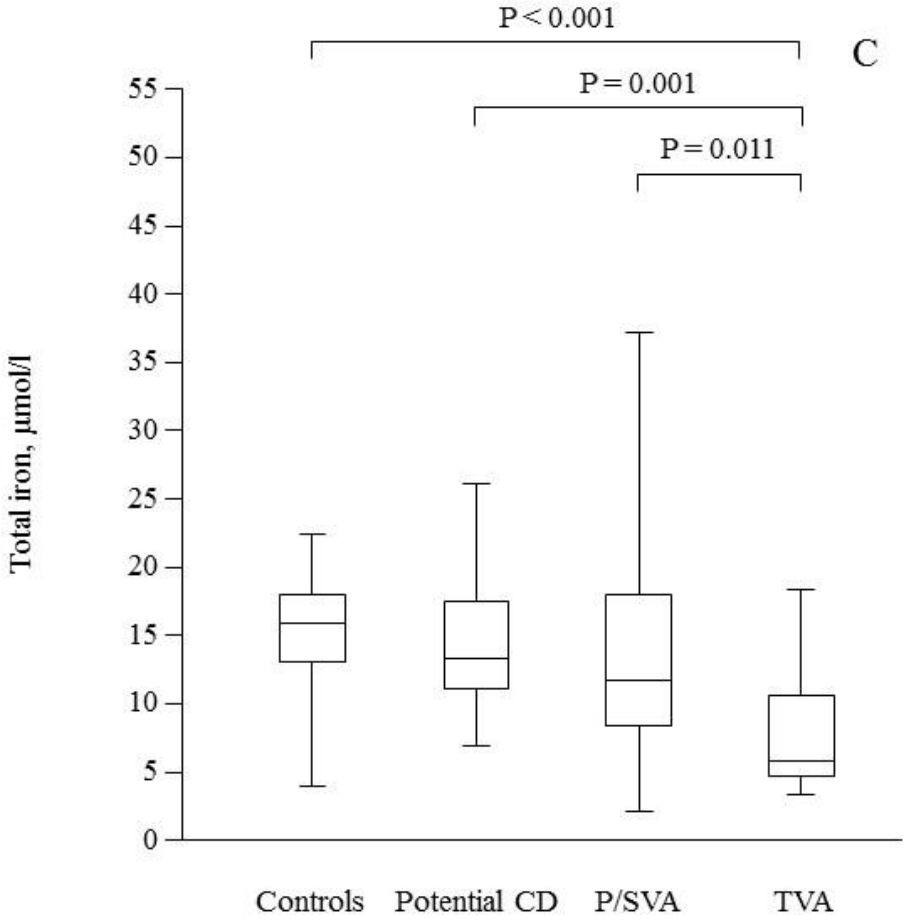
Table 3. Percentages of anemia and abnormal iron parameters in 23 non-celiac controls, 19 children with potential celiac disease and 83 with villous atrophy celiac disease.

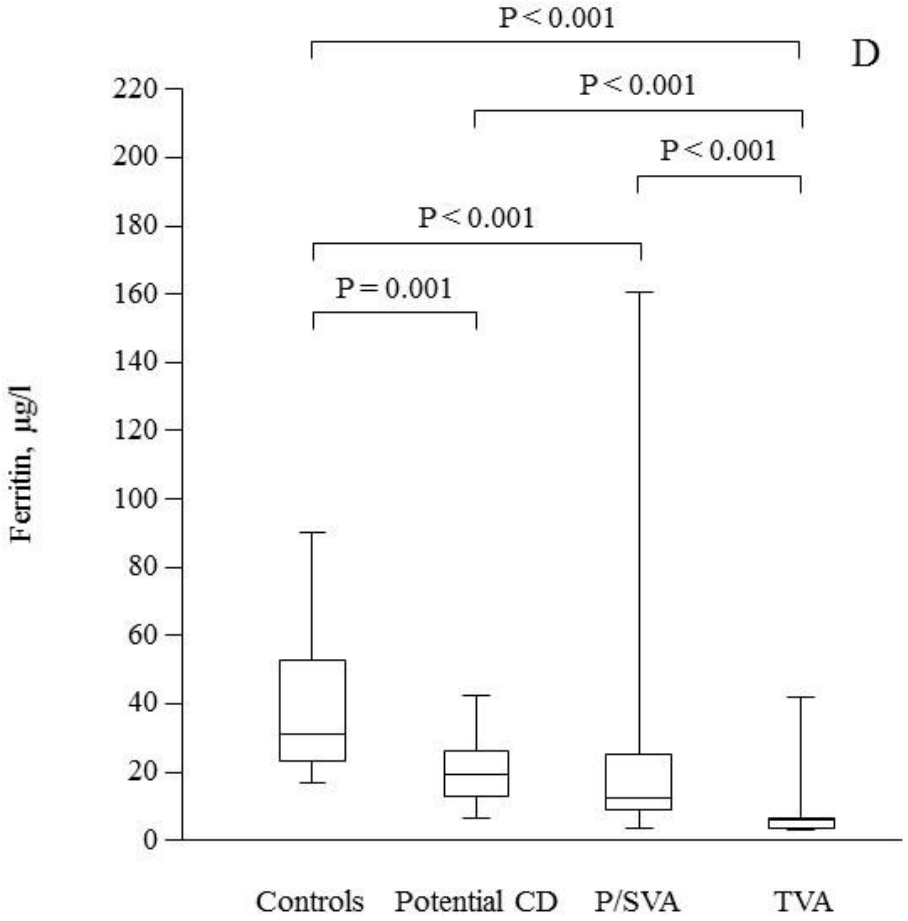
	Non-celiac controls n=23	Potential celiac disease n=19	Celiac disease n=57		P value
			P/SVA n=67	TVA n=16	
	%	%	%	%	
Anemia	0.0	15.3	22.4	62.5	**<0.001**
Low total iron	4.8	0.0	13.6	50.0	**<0.001**
High TfR1	4.8	15.8	20.4	46.7	**0.002**
Low ferritin	0.0	21.1	35.2	86.7	**<0.001**
Low transferrin saturation	9.5	11.1	40.5	71.4	**<0.001**
Low vitamin B12	0.0	0.0	0.0	0.0	1.000
Low erythrocyte folate	0.0	0.0	2.3	0.0	1.000

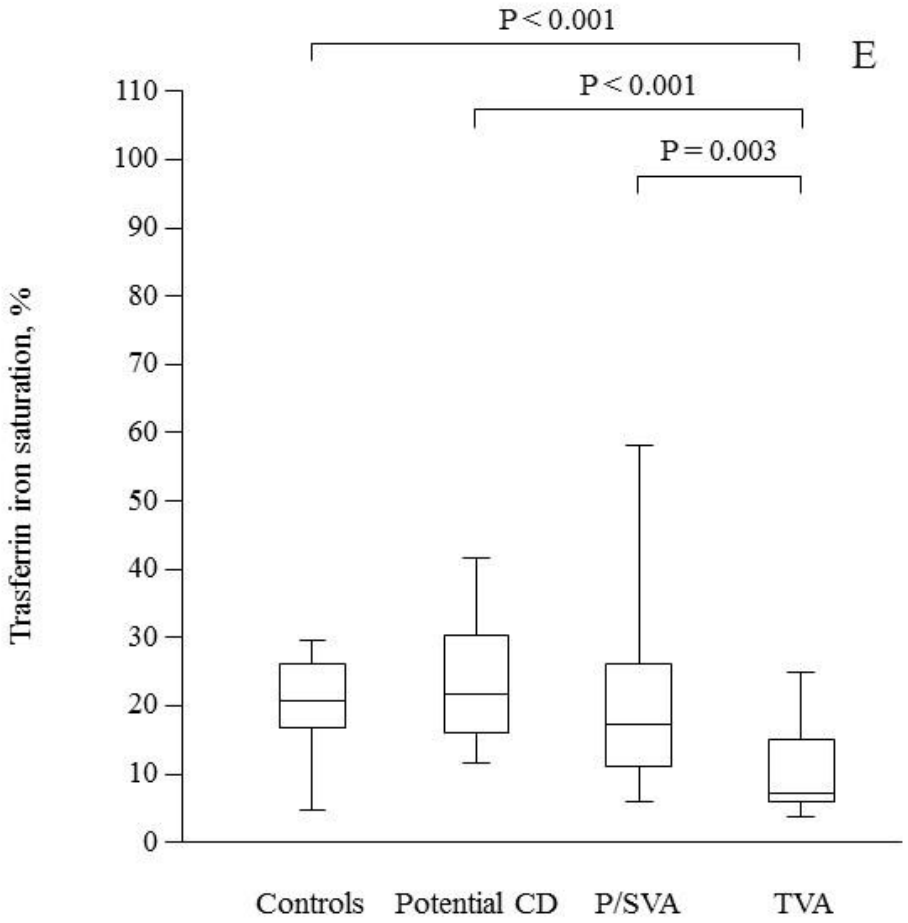
TfR1, transferrin receptor 1
Low denotes values below reference values. High denotes values above reference values.

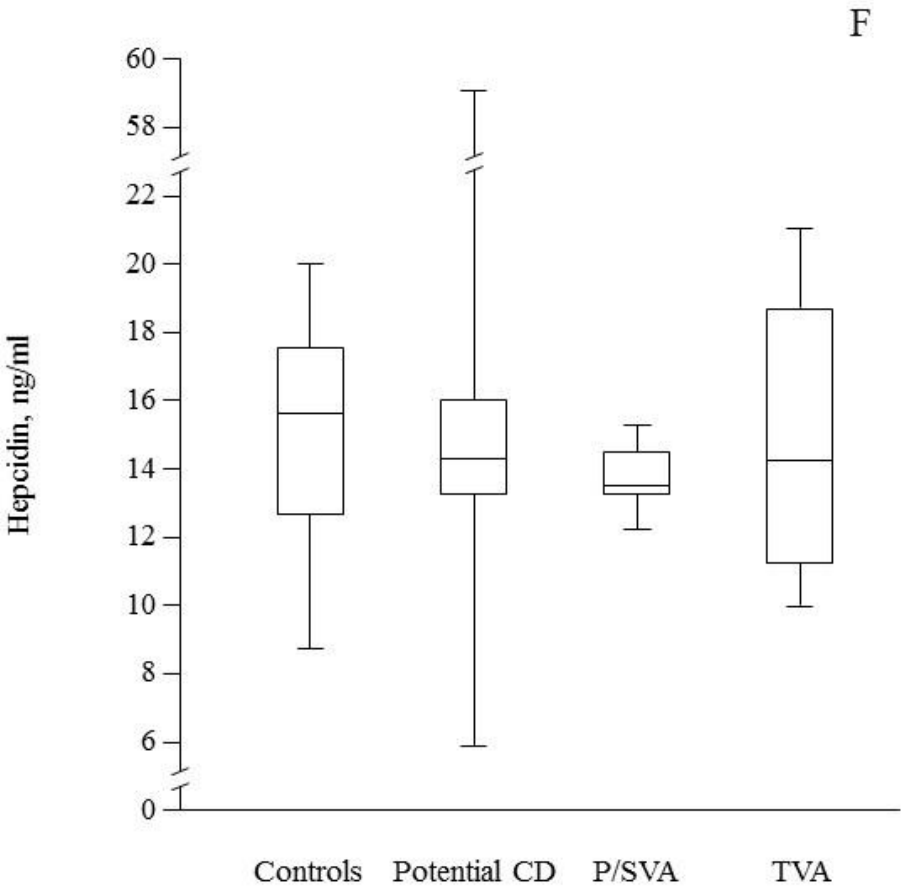

P < 0.001
A
P < 0.001
P = 0.001
190
180
170
160
150
140
130
120
110
100
90
0
Hemoglobin, g/l
Controls
Potential CD
P/SVA
TVA

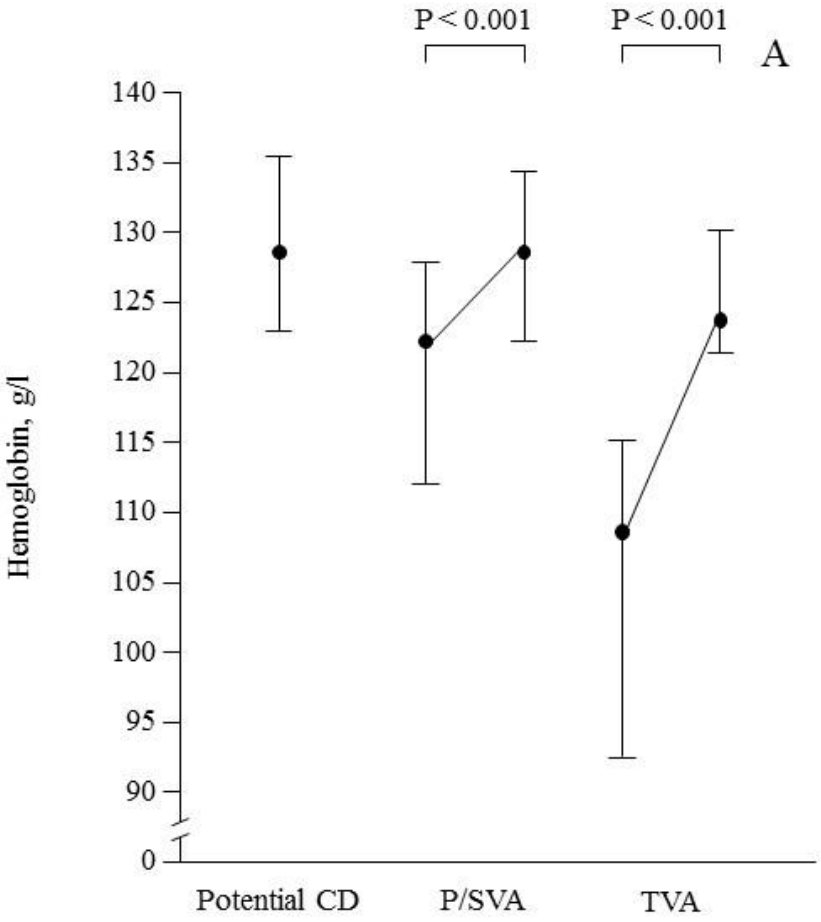

P = 0.008
B
P = 0.001
P = 0.002
30
28
26
24
14
12
10
8
6
4
2
0
Transferrin receptor 1, ng/l
Controls
Potential CD
P/SVA
TVA

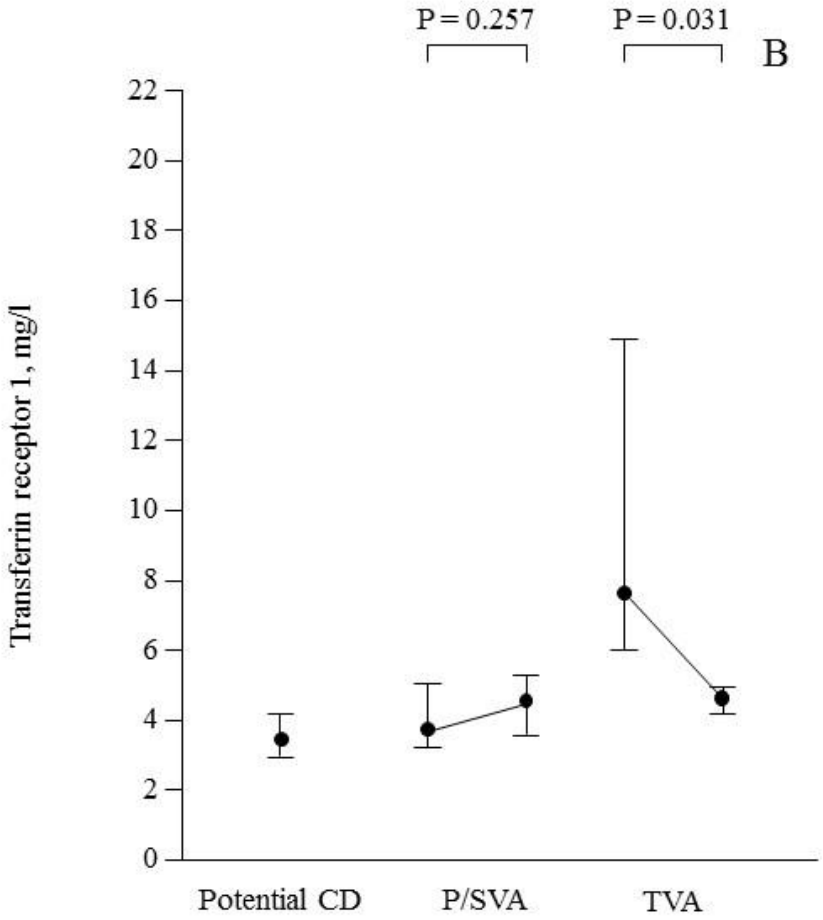

P < 0.001
C
P = 0.001
P = 0.011
55
50
45
40
35
30
25
20
15
10
5
0
Total iron, µmol/l
Controls
Potential CD
P/SVA
TVA

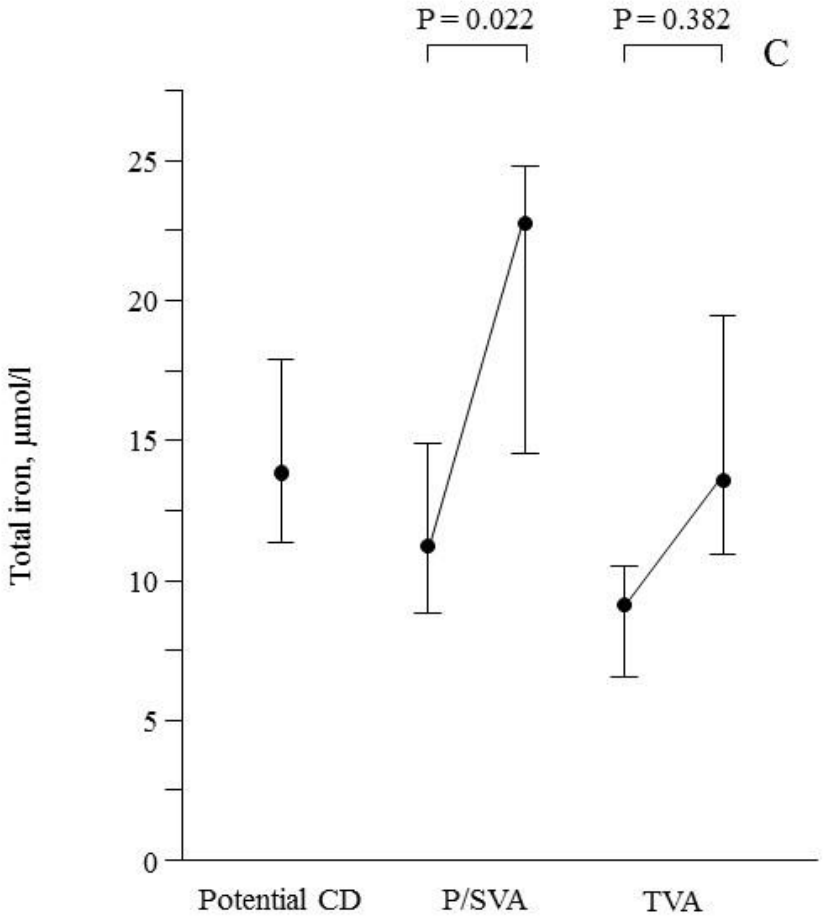

P < 0.001
D
P < 0.001
P < 0.001
P < 0.001
P = 0.001
Ferritin, µg/l
220
200
180
160
140
120
100
80
60
40
20
0
Controls
Potential CD
P/SVA
TVA

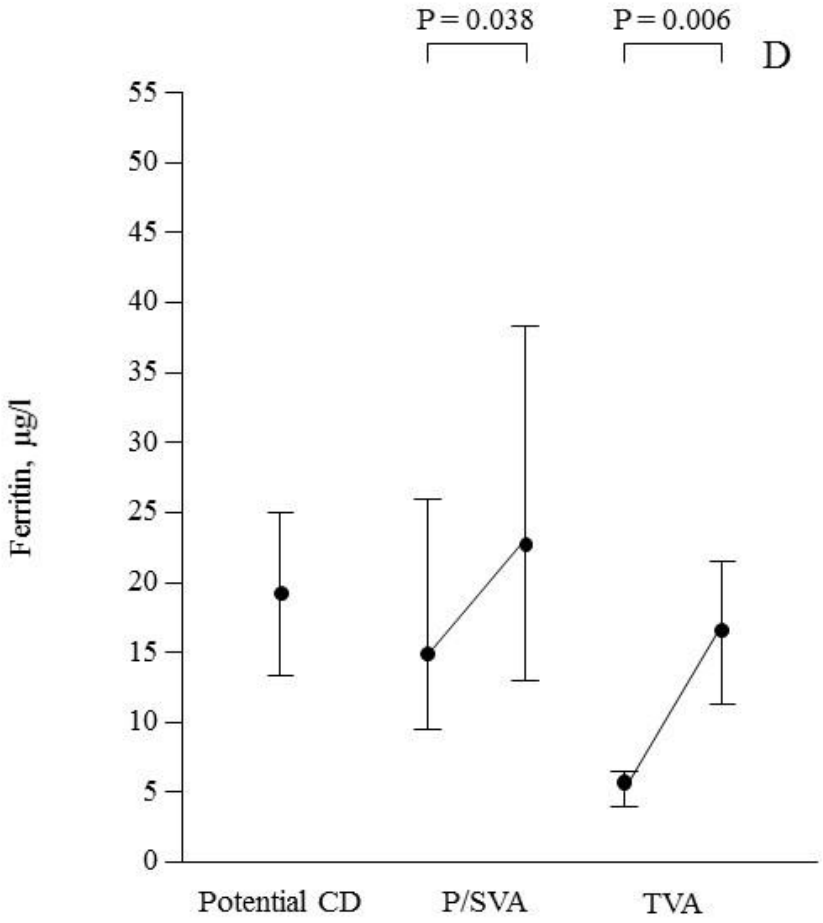

P < 0.001
E
P < 0.001
P = 0.003
Trasferrin iron saturation, %
110
100
90
80
70
60
50
40
30
20
10
0
Controls
Potential CD
P/SVA
TVA

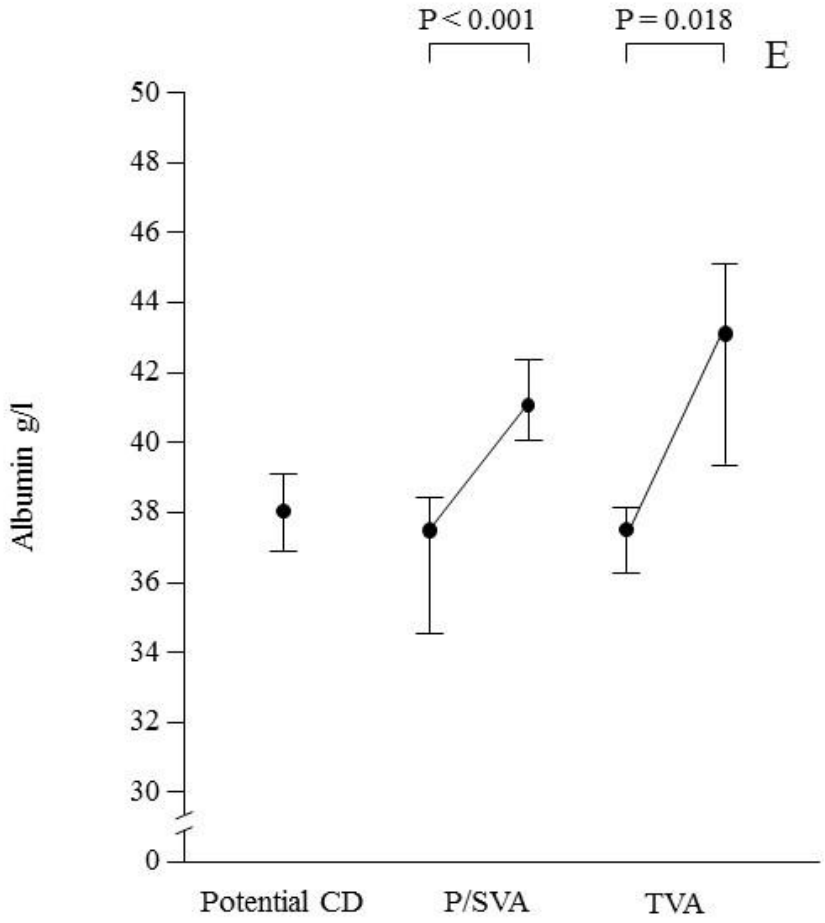

F
Hepcidin, ng/ml
60
58
22
20
18
16
14
12
10
8
6
0
Controls
Potential CD
P/SVA
TVA


P < 0.001
P < 0.001
A
Hemoglobin, g/l
140
135
130
125
120
115
110
105
100
95
90
0
Potential CD
P/SVA
TVA


P = 0.257
P = 0.031
B
Transferrin receptor 1, mg/l
22
20
18
16
14
12
10
8
6
4
2
0
Potential CD
P/SVA
TVA


P = 0.022
P = 0.382
C
25
20
15
10
5
0
Total iron, µmol/l
Potential CD
P/SVA
TVA


P = 0.038
P = 0.006
D
55
50
45
40
35
30
25
20
15
10
5
0
Ferritin, µg/l
Potential CD
P/SVA
TVA


P < 0.001
P = 0.018
E
Albumin g/l
50
48
46
44
42
40
38
36
34
32
30
0
Potential CD
P/SVA
TVA